Docket No.: 20526 US (C038435/0111695)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Maurice Raymond HICKLING

Serial No.: 09/734,803

Filed: December 12, 2000

For: **HAIR COLORANT COMPOSITION CONTAINING PHYTANTRIOL**

Examiner: S. Gollamudi

Art Unit: 1616

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF JÜRGEN VOLLHARDT, Ph.D. UNDER 37 C.F.R. §1.132

Sir:

I, Jürgen Vollhardt, Ph.D., a citizen and resident of Germany, hereby declare as follows:

1. I received a doctorate in Organic Chemistry in 1990 from the Technical University of Darmstadt, Germany.

2. From 1991 to 1999, I was employed by Dragoco AG in Germany. While employed there, I held positions as Department Manager of the Cosmetic Ingredient Laboratory and as Laboratory Manager.

3. From 1999 to 2003, I was employed by Dragoco Inc. in Totowa, New Jersey. From 1999-2000, I held the position of Vice President R&D Cosmetic Ingredients, Head of Technology. Thereafter, I was the Vice President Business Unit Cosmetic Activities.

4. I am presently employed by Roche Vitamins Ltd., Kaiseraugst, Switzerland as Deputy Head of R&D Cosmetics. Since receiving my doctorate, I have been working on or supervising research and development projects in the cosmetics field and I am intimately familiar with the chemistry underlying the above-identified application.

5. I understand that an Office Action has issued in the above-identified application. I further understand that claims 1, 3-6 and 9-12 have been rejected over Ribier *et al.*, U.S. Patent No. 5,756,108 ("Ribier") alone or in combination with other documents. I have reviewed Ribier, which discloses compositions containing an oily phase in an aqueous phase dispersion that is stabilized by cubic gel particles. Such compositions are disclosed to have use in the cosmetic, dermatological and pharmaceutical fields. (Abstract) More particularly, Ribier discloses:

> a composition in the form of a dispersion comprising:
> (a) from 60 to 98% by weight of an aqueous phase, and
>
> (b) from 2 to 40% by weight of an oily phase, ***said oily phase being dispersed in said aqueous phase and stabilized by using cubic gel particles***, said particles being essentially formed of:
>
> (i) 0.1 to 15% by weight, relative to the total weight of the composition, of at least one component selected from the group consisting of 3,7,11,15-tetramethyl-1,2,3-hexadecanetriol or phytanetriol, N-2-alkoxycarbonyl derivatives of N-methylglucamine and unsaturated fatty acid monoglycerides, and
>
> (ii) 0.05 to 3% by weight, relative to the total weight of the composition, of a dispersing and stabilizing agent, said agent being selected from the group consisting of surface-active agents which are water-soluble at room temperature, containing a linear or branched, saturated or unsaturated fatty chain having from 8 to 22 carbon atoms. (Col. 1, line 56 - col. 2, line 7.)

6. Ribier acknowledges that emulsions of an oily and an aqueous phase have been previously produced, but criticizes such systems as lacking stability over time and for containing emulsifying agents/surfactants that may cause skin irritation at the concentrations employed. (Col. 1, lines 15-32.) Ribier discloses that its dispersions are superior to such prior art compositions because they are "particularly stable and non-irritant" because of the presence of the cubic gel particles.

> It has now been observed, surprisingly and unexpectedly, that it is possible to obtain dispersions of an oily phase in an aqueous phase, which are particularly stable and non-irritant, using a very large variety of oils, ***by using cubic gel particles containing a low proportion of a water-soluble surface-active agent*** containing a fatty chain. The dispersions thus obtained moreover have particularly satisfactory sensory qualities. (Col. 1, lns. 33-40).

7. As summarized in Table 1 below, all of the compositions exemplified by Ribier contain more of the lipid phytantriol compared to the dispersing agent.

Example No.	Phytantriol (g)	Dispersing Agent (g)	Ratio of Phytantriol to Dispersing Agent
1	3.00	.95	3.15 : 1
2	2.97	.95	3.12 : 1
6	2.97	1.00	2.97 :1
7	2.97	1.00	2.97 : 1
8	1.96	0.75	2.61 : 1
10	0.3	0.1	3 : 1

Table 1. Ratio of phytantriol to dispersing agent in Ribier examples.[1]

[1] Examples 3-5, comparative examples, do not contain phytantriol. Example 9 includes both phytantriol (0.27 g) and N-2-hexyldecyloxycarbonyl-N-methylglucamine (2.43 g) and 0.5 g of the dispersing agent. This provides a phytantriol:dispersing agent ratio of 5.46:1. These examples are not included in the table.

Ribier is thus consistent with my understanding that cubic gel particles can only be formed when the ratio of polar lipid:dispersing agent is greater than 1:1.

Structure And Physical Characteristics Of Cubic Gel Particles

8. Cubic gel particles, such as those disclosed by Ribier, are formed from cubic gels. Cubic gels are formed only through the aide of high energy mixing. Such gels are characterized by their high viscosity. Cubic gels may be broken up into gel particles also with the aide of high energy mixing and dispersed in water with a dispersing agent, such as polysorbate. Cubic gel particles have a characteristic cubic gel conformation as visualized by X-ray diffraction. This characteristic structure is maintained even when the cubic gel particles are dispersed in water. *See e.g.*, Sven Engström, *Drug delivery from cubic and other lipid-water phases*, **Lipid Technology** Vol. 2 No. 2 (April 1990) p. 42-45, a copy of which is attached as Exhibit 1.

9. Thus, cubic gel particles are a special arrangement of polar lipids in water. As noted above, to form cubic gel particles, care must be taken in selecting the ratio of the polar lipid:water and how these components are combined. It is critical that the ratio of polar lipid:water be greater than 1:1, such as approximately 7:3, and that the two components are mixed using a high energy mixer. Cubic gel particles will not form unless a high energy mixer is used. Nor will cubic gel particles form if a ratio of polar lipid:water of less than 1:1 is used.

10. The size of cubic gel particles formed from a cubic gel is related to the energy of the high energy mixer. It is my understanding that the 0.48 μm diameter cubic gel particles obtained by Ribier approaches the practical lower limit for cubic gel particles in view of the energy constraints of current state-of-the-art high energy mixers.

11. To confirm the observations set forth above, I supervised and directed an experiment described in ¶¶12-14 below comparing the process for forming dispersions with cubic gel particles according to the method disclosed in Ribier with the process for forming the claimed compositions in the present application. The products formed from these respective methods were also compared. As summarized in Table 2 below, the dispersions containing the cubic gel particles of Ribier are physically different than the micellular compositions of the present invention with respect to, at least, viscosity, ease of incorporation into water, particle size, appearance of the final products and structure.

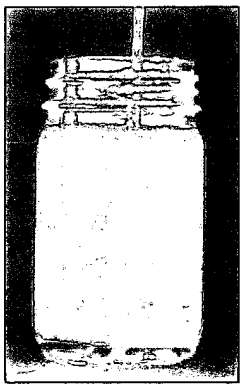
Table 2

	Cubic gel (Phytantriol+ water ratio 7:1)	**Micelles in water (phytantriol solubilized in polysorbate ratio 1:4) added in water**
Viscosity	Very high, is characteristic of the thick gel formed when mixing phytantriol with water.	Very low. No gel formation at any step of the preparation. The mixture of phytantriol and polysorbate leads to no gel formation.
Incorporation into water	Difficult: Gel has to be broken into smaller particles (high energy needed to achieve small particle size) and dispersed in water using polysorbate	Easy: Micelles are formed instantly upon adding the premix (phytantriol + polysorbate) to water, with minimal energy (magnet stirrer)
Particle size	Measured: 85 µm (dispersion of gel in water with polysorbate, using conventional high energy mixing). The dispersed gel particles are bigger then the micelles present in the subject application	Measured: 0.1 µm Size of micelles is independent of energy input during the mixing (in this case, no highly viscous gel to break up)
Appearance	The dispersion of cubic gel particles in water with polysorbate provides an opaque dispersion	The dispersion of phytantriol in water in the micelles formed by polysorbate provides a transparent dispersion
Identification	Specific X-ray signature (cubic symmetry)	No cubic symmetry patterns observed by X-ray analysis

Preparation Of Cubic Gel Particles - Ribi r

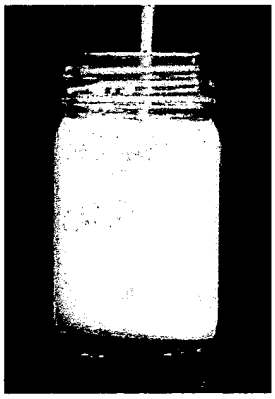
12. 1.0 g phytantriol was mixed with 0.43 g water using a conventional high energy mixer (Ultraturrax T25), which formed a highly viscous and transparent gel. 1.43 g of this gel was added to 18.57 g of an aqueous solution containing 2% polysorbate (Tween 40) (0.37 g). This equates to a phytantriol:dispersing agent ratio of 2.70:1. The mixture was homogenized at room temperature using a conventional high energy mixer (Ultraturrax T25) at 35,000 rpm for 5 min. The homogenization was repeated four times. The resulting dispersion formed a milky, highly viscous cubic gel composition with a mean particle size of 85 µm as shown in Figure 1 below.

Figure 1



13. To a cubic gel composition prepared according to ¶12, an oil phase was added and mixed with the high energy mixer. The resulting dispersion formed an opaque suspension as shown in Figure 2 below.

Figure 2



Preparation Of Micellular Compositions - Present Invention (Example 1)

14. 0.2 g phytantriol was mixed with 0.8 g polysorbate (Tween 20) at room temperature under slow agitation (conventional mixing). This equates to a phytantriol:dispersing agent ratio of 0.25:1. This mixture was added to 99.0 g of water and mixed under slow agitation at room temperature with a water soluble dye (Arianor Red) until a homogeneous preparation was obtained. The resulting dispersion formed a translucent composition with a mean particle size of 0.1 µm as shown in Figure 3 (left-hand jar). For comparison, the right-hand jar is included, which is a cubic gel particle composition prepared according to ¶12.

Figure 3

Based on the foregoing, it is clear that the Ribier dispersions containing cubic gel particles are physically different than the compositions according to the present invention.

15. Based on my knowledge and experience, and in view of the results presented above, I have concluded that it is impossible to form cubic gel particles from a composition in which the amount of dispersing agent is greater than the amount of phytantriol. Cubic gel particles may be produced from a composition containing phytantriol and a dispersing agent, only if the amount of phytantriol is greater than the amount of dispersing agent and a high energy mixer is used. Such cubic gel particles, however, are physically different than the compositions according to the present invention. For example, the cubic gel particles of Ribier form opaque emulsions/suspensions and are limited to a practical diameter of at least approximately 0.5 μm compared to the compositions of the present invention, which form translucent preparations with a significantly smaller particle diameter of about 0.1 μm. It is therefore my opinion that Ribier does not -and cannot- disclose or suggest a composition formed of cubic gel particles of phytantriol wherein the amount of dispersing agent is greater than the amount of phytantriol.

I d clare further that all statements made herein of my own knowledg are true and that all statements made on information and b lief are believed to be true, and further that these statements were made with the knowledg that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: Kaiserslautern, 10/28/03

Jürgen Vollhardt, Ph.D.

CUBIC LIPID-WATER PHASES

DRUG DELIVERY FROM CUBIC AND OTHER LIPID-WATER PHASES

Sven Engström

This article presents the results of some work dealing with lipids as drug carriers performed by the Drug Delivery Group at the Chemical Center in Lund. The research has as its starting-point the rich variety of structures emerging in systems consisting of polar lipids and water which have been analyzed by various techniques at the Department for some time.

Polar lipids in water - a matter of organisation

Polar lipids are amphiphilic molecules, that is to say they both like and dislike water. Thus, when placed in a water solution they have to associate in one way or another forming different kinds of aggregates. Perhaps the most well-known aggregate formed in water is the spherical micelle consisting of typically 50-100 lipid molecules arranged so that their hydrocarbon tails (the *hydrophobic* part) form the interior of the micelle, and the polar head groups (th *hydrophilic* part) act as a shield against the surrounding water (Fig.1).

The micelle, however, is only one of many aggregate types formed. One also finds rod-shaped micelles as well as micelles of the reversed type (L_2), where water forms the interior. A number of liquid crystalline structures are also normally found in polar lipid-water systems, giving rise to equilibrium phases. These include hexagonal phases of the normal (H_1) and the reversed (H_{II}) type and the lamellar phase (L_α). The lamellar structure is the origin of liposomes, consisting f spherical shells of lipid bilayers. These are frequently studied and used in the context of drug release, for example in chemotherapy of cancer.

A less well-known liquid crystalline phase is the *cubic* phase (C). The name comes from the fact that its X-ray diffraction pattern reveals cubic symmetry. The structure of the cubic phase varies depending on the system. The simplest structure consists of spherical micelles close-packed in a cubic pattern. Such a cubic phase is said to be *water-continuous* and *oil-discontinuous*. However, there are other types of cubic phases and one of them will be discussed in more detail below.

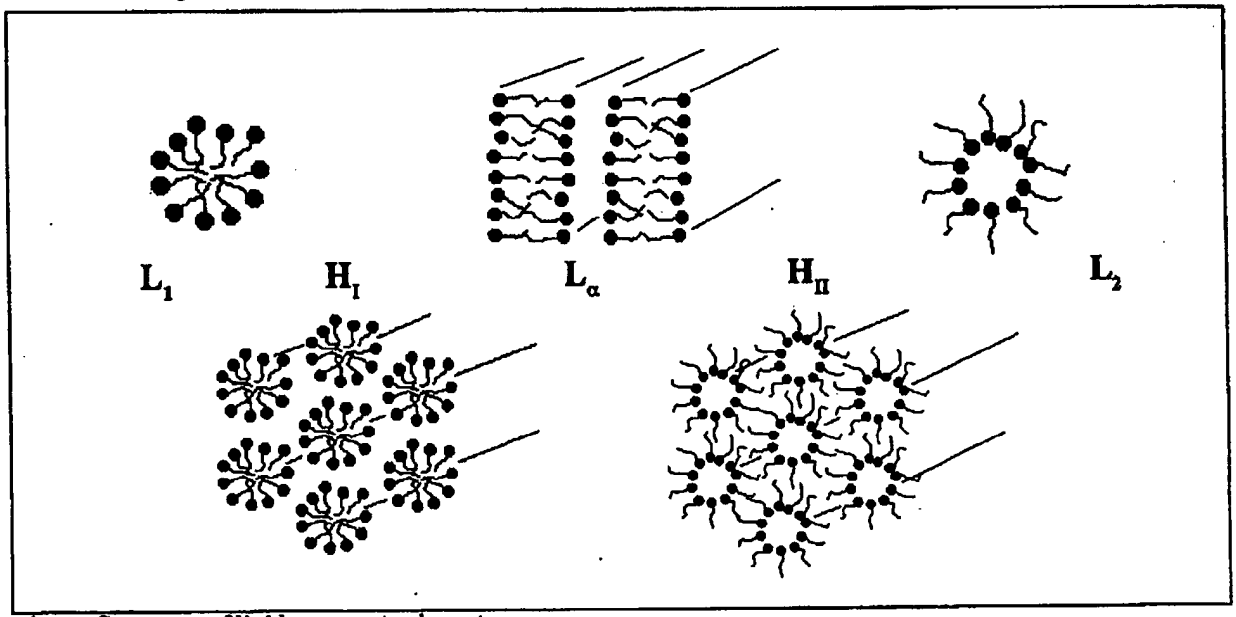


Fig. 1 Structure of lipid aggregates in water

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M noolein

Monoolein (or glyceryl monooleate) is a polar lipid which swells in water, giving rise to several of the phases m ntioned

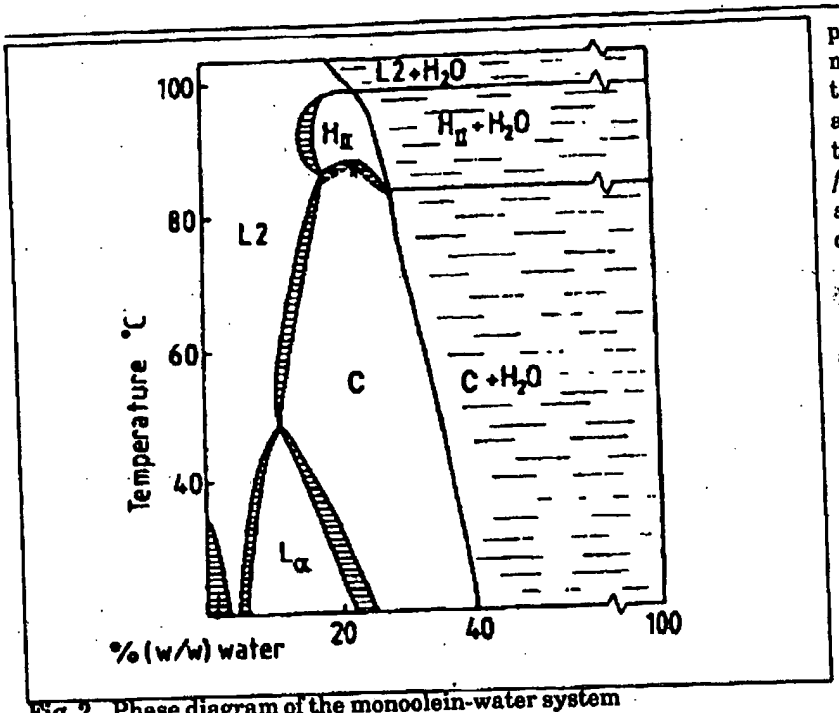

Fig. 2 Phase diagram of the monoolein-water system

above including the cubic phase. A representation of the system is given in Figure 2, where the phase diagram is shown. The diagram reveals a number of one-phase regions - L_2, L_α and C - at normal temperatures, and an H_{II} phase at higher temperatures. In order to illustrate the rheological properties of the system, one can add water to monoolein. (The reader should be aware of the fact that the time taken for the various mixtures to reach equilibrium may be a couple f hours.)

Monoolein itself is a waxy material at room-temperature and melts in pure form at 36 °C (normally we use a monoolein-rich blend of monoglycerides originating from, for example, sunflower oil). If we add a small amount of water to monoolein at, say, 37 °C this water will form reversed micelles in the lipid. This phase, L_2, is like a liquid oil. Adding more water, the system enters the lamellar phase region, L_α, a phase which is mucous-like and birefringent. With even more water, the system becomes very viscous and the resulting phase is glass-clear - the cubic phase. Hence, the more water added, the higher the viscosity! At high water content, the cubic phase is in equilibrium with essentially pure water (the monoolein solubility in water is about 10^{-6} M).

The structure of the monoolein-water cubic phase is more complicated than the simple body-centered lattice. It has been shown to consist of a curved bilayer extending in three dimensions, separating two congruent networks of water channels. This cubic phase is, therefore, said to be *bicontinuous*. The surface generated by the plane in the middle of the monoolein bilayer forms a so-called *infinite periodic minimal surface*. This implies that each point on such a surface is a saddle point with zero *average* curvature. A sketch of the structure is given in Figure 4. The water pore diameter is about 5 nm (1 nm = 10^{-9}m) when the cubic phase is fully swelled.

The survival of the cubic phase in excess water, its structural properties, as well as the fact that monoolein is subject to lipolysis due to different kinds of esterase activity in different tissues, add up to make the cubic phase an *in situ forming biodegradable matrix system* and, as such, a potential candidate for drug delivery.

Drugs in the cubic phase

The cubic phase, with its lipid and water domains, may in principle solubilise both water- and lipid-soluble substances, as well as molecules with pronounced amphiphilic characters. That this is the case is seen in Table 1, which shows examples of compositions giving rise to cubic phases (the monoolein / water ratio is about 65 / 35). These cubic phases contain compounds of different polarity - from *NaCl* on the one hand, via the surface active local anesthetic *lidocaine*, to the lipophilic polypeptide *gramicidin* on the other; and molecules of different sizes - from the oligopeptide *desmopressin*, through *insulin*, to the protein *bovine serum albumin* (*BSA*).

Table 1.
Cubic phases formed from substances with different polarity and size

Compound	M_w	% (w/w)	ref.
sodium chloride	58	0.9	
lidocaine	270	5	4
gramicidin	1141	6	
desmopressin	1069	4	
insulin	6000	4	
bovine serum albumin (BSA)	67000	18	3

In one of our projects, the influence of an amphiphilic drug, lidocaine, on the cubic phase is investigated. Lidocaine is a local anesthetic with surface active properties and one should, therefore, expect that this molecule will interact with lipid aggregates. Moreover, lidocaine exists in a charged and an uncharged form at most physiological conditions since its $pK_a \approx 8$. The effects on the cubic phase are shown in the phase diagrams (at 20 °C and 37 °C, respectively) in Figure 3. The right hand corner in each diagram represents a cubic phase with 65% (w/w) monoolein in water.

CUBIC LIPID-WATER PHASES

The phase diagrams show that as the content of the salt form of lidocaine (L:HCl) increases, the cubic phase is transformed into a lamellar, or L_α, phase. When the base form (L) is added, on the other hand, phases of the reversed types are formed - H_{II} and L_2. With the structure of the monoolein-water cubic phase in mind, it is rather obvious that the lipid packing in the bilayer is influenced when compounds like lidocaine interact with it, eventually resulting in molecules is the same but the polar parts differ since the salt form is positively charged. Both types of lidocain will probably be embedded in the monoolein bilayer with their polar parts at the lipid-water interface. However, the salt form demands a larger area for its charged polar head group in comparison with the uncharged polar head group of the base form. This results in the tendency to curve the lipid-water interface in one way for L:HCl, and the opposite reversed types of phases (L_2 and H_{II}) grow at the expense of the normal types (L_α). This can be explained by the increasing chain mobility which in turn increases the apparent hydrocarbon volume. It is obvious from the phase diagrams in Figure 3 that the phase behaviour has been determined carefully. However, once done, it may give rise to new exciting ideas about how to use the system f r drug delivery. It should be pointed out that the phase behaviour found

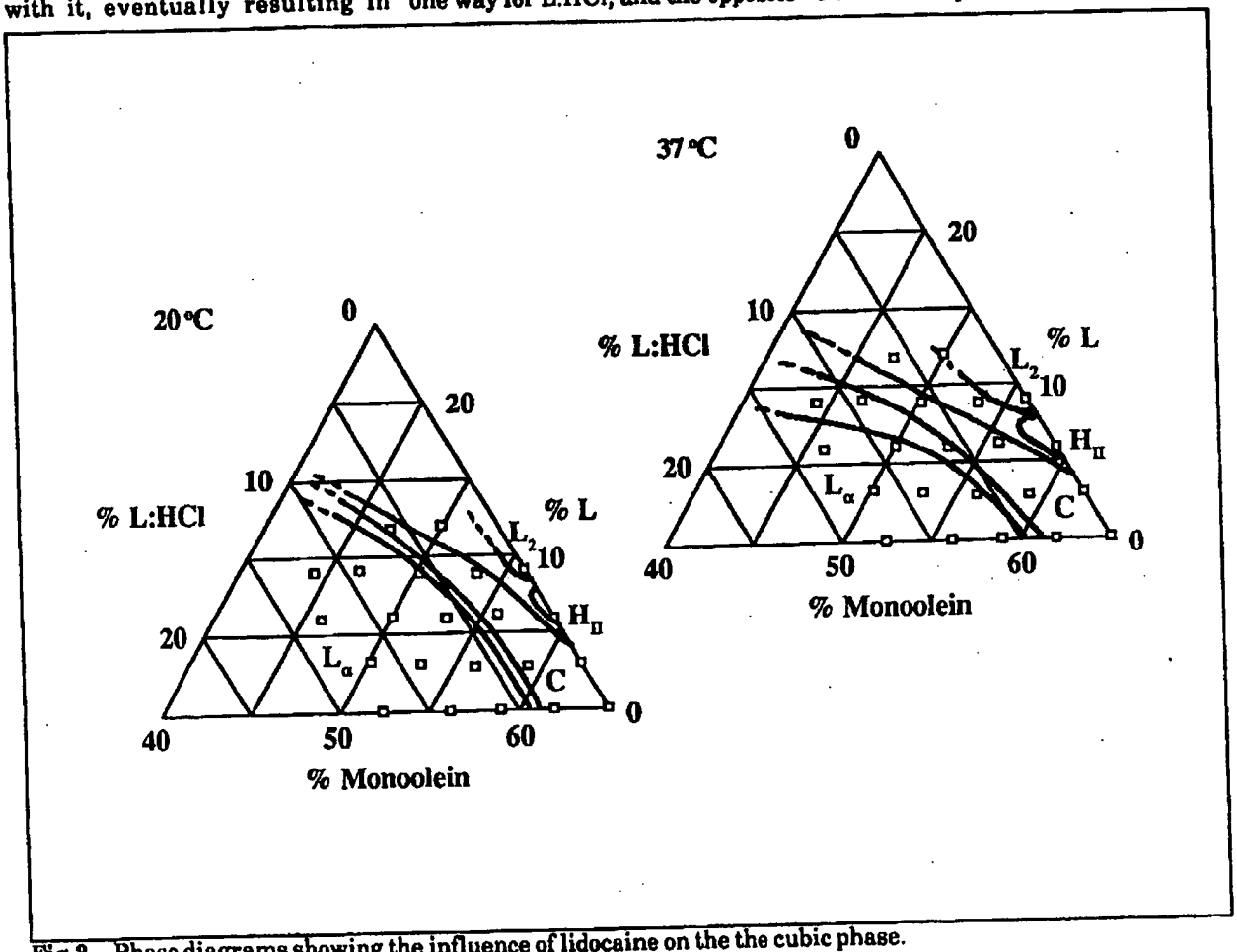

Fig.3. Phase diagrams showing the influence of lidocaine on the the cubic phase.

phase transitions. It turns out that the phase behaviour may be rationalized by making use of a combination of geometrical parameters like polar head group area, hydrocarbon volume and chain length f the amphiphilic substances (the so-called critical packing parameter).

In th lidocaine case, the effects on the cubic phase can be explained by the difference in packing b havi ur of the two lidocain forms. The lipophilic part of th way for L. The validity of the packing concept is further supported by the fact that if both lidocaine forms are mixed, which is the most probable situation under biological conditions, the cubic phase exists at a certain range f mixing ratios, since the effects then cancel.

The changes in the phase behaviour as a result of the temperature increase from 20 °C to 37 °C are given in Figure 3 as w ll. Th general tr nd is that the for lidocaine should be qualitatively similar for many other amphiphilic drugs.

The Cubosome formulation - a dispersed cubic phase

The rheological property of th cubic phase, its high viscosity, causes problems in many cases, since it is difficult to handle. One important part of ur res arch is therefore dir cted towards devel ping dispersion methods for the cubic phase. Th goal was t

make a cubic phase dispersion with a particle-size distribution suitable for intravenous injection. We have so far succeeded in making cubic phase dispersions with that pr p rty by making use f an amphiphilic polymer as the emulsifier. We denote the resulting dispersion a Cubosome™ of insulin through rat nasal mucosa. At present, we are focusing our interest on th biodistribution f the Cubosome particles in different tissues after intravenous injecti n. This w rk is done in collaboration with pharmacologists and physicists. pharmaceutical products based on liposomes on the market. One reason for this is the costs associated with lipids of acceptable and reproducible quality. Perhaps the demands put f rward by the new peptide and pr tein drugs for new delivery systems will improve the situation.

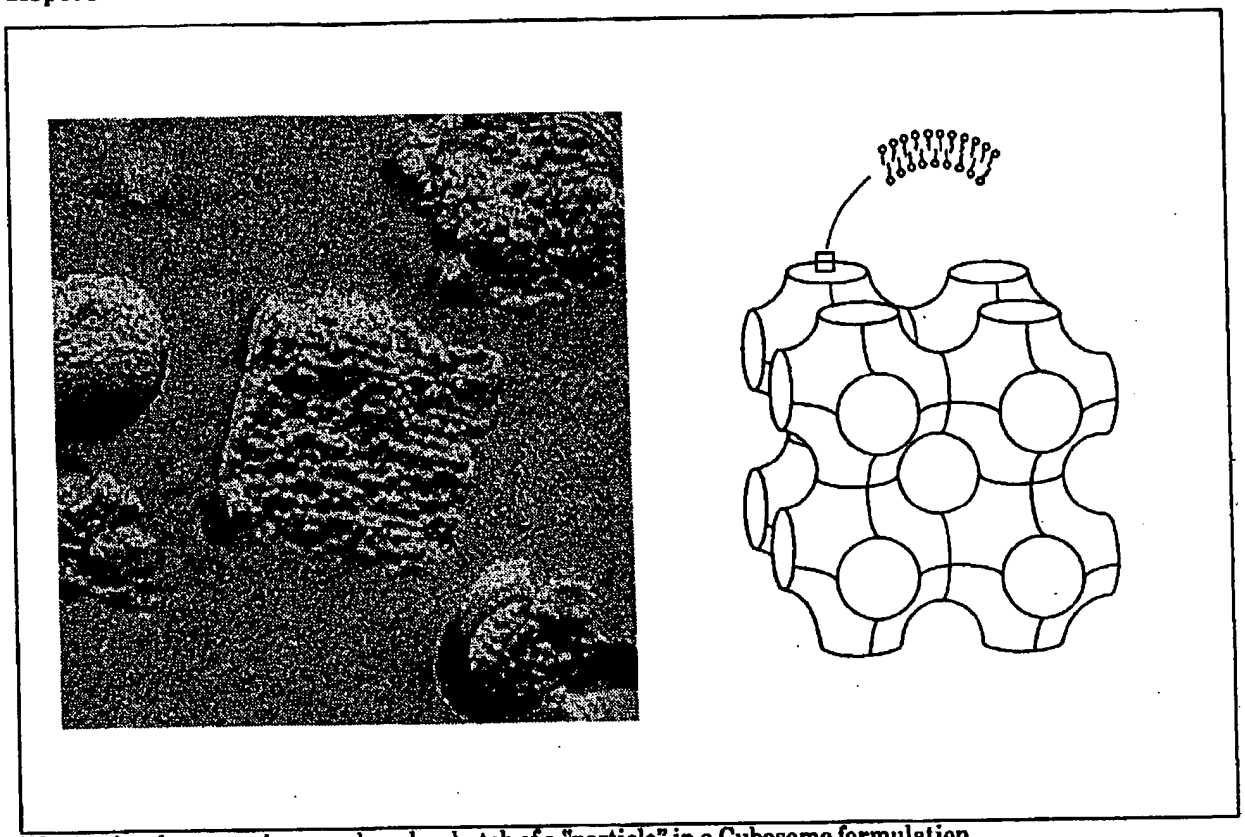

Fig.4. An electron micrograph and a sketch of a "particle" in a Cubosome formulation

formulation, and have begun to investigate both its release and biological properties (Fig.4).

The most well-known pharmaceutical lipid dispersions to date are the *oil-in-water emulsions* and the *liposomes*. A Cubosome dispersion has some unique properties in comparison with them, for example the internal structure of the "particle". The possibility of incorporating drugs of various kinds seems greater in the Cubosome formulation than in the liposome and in the emulsion (only lipid-s luble drugs). Two preliminary *in vivo* tests hav revealed that the dispersed cubic phase has the capability of (i) maintaining a high plasma l vel of an ligopeptide for several hours, and (ii) promoting the penetration

Conclusions

Although polar lipids have been used for a long time in the pharmaceutical industry, many of their properties in aqueous solution have still not been utilized in the context of drug delivery. Among the liquid crystalline phases, the lamellar phase in the dispersed state as liposomes has received the greatest interest, but there are few

Acknowledgements

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